

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning documents *will not* correct images,  
please do not report the images to the  
Image Problem Mailbox.**

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



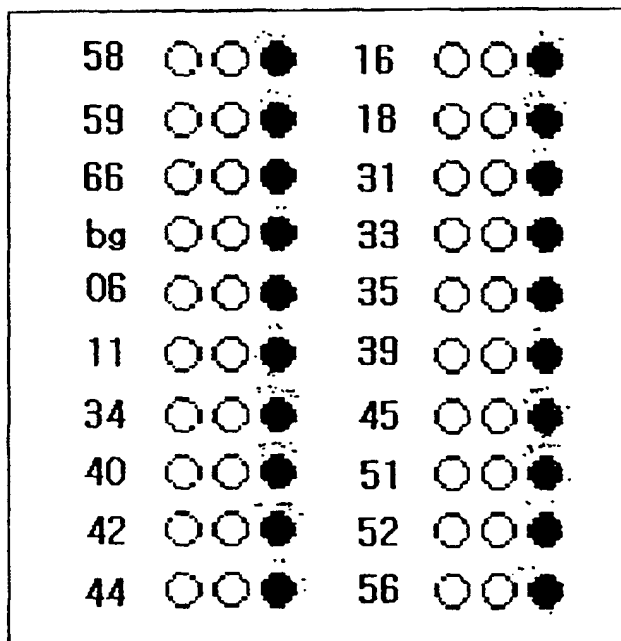
(43) International Publication Date  
20 September 2001 (20.09.2001)

PCT

(10) International Publication Number  
**WO 01/68915 A1**

- (51) International Patent Classification<sup>7</sup>: C12Q 1/68 (74) Agent: LEE, Han-Young; Seowon Building, 8th Floor, 1675-1 Seocho-dong, Seocho-gu, Seoul 137-070 (KR).
- (21) International Application Number: PCT/KR00/01213
- (22) International Filing Date: 26 October 2000 (26.10.2000)
- (25) Filing Language: Korean
- (26) Publication Language: English
- (30) Priority Data:  
2000/13161 15 March 2000 (15.03.2000) KR
- (71) Applicant (for all designated States except US):  
**BIOMEDLAB CORPORATION** [KR/KR]; Dongsung Building, 4th Floor, 1-49 Dongsung-dong, Jongno-gu, Seoul 110-510 (KR).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **PARK, Tae-Shin** [KR/KR]; 81-4 Yeonhui 2-dong, Seodaemun-gu, Seoul 120-112 (KR). **KIM, Sung-Keun** [KR/KR]; 77-27 Sang-gae 3-dong, Nowon-gu, Seoul 139-203 (KR). **KIM, Jin-Hee** [KR/KR]; 102-19 Amsa-dong, Gangdong-gu, Seoul 134-050 (KR). **PARK, Mi-Sun** [KR/KR]; 479-1 Mangwon-dong, Mapo-gu, Seoul 121-230 (KR).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:  
— with international search report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: GENOTYPING KIT FOR DIAGNOSIS OF HUMAN PAPILLOMAVIRUS INFECTION



(57) Abstract: The present invention relates to a genotyping kit for diagnosing patients infected with human papillomavirus (HPV), and a method for diagnosis of HPV infection by genotyping specimen DNA isolated from the patients using the said kit. The genotyping kit of the invention comprises a DNA chip with probes that have nucleotide sequences complementary to DNA of HPV, primers for amplifying the DNA of a sample by PCR, and means for labeling hybridized sample DNA with the DNA chip. The method for diagnosis of HPV infection comprises the steps of amplifying DNA obtained from a sample using the primers of the kit, applying the amplified DNA to the DNA chip and hybridizing the amplified DNA and the probes of the DNA chip, and detecting DNA bound on the surface of the DNA chip by labeling hybridized DNA. In accordance with the invention, the genotyping kit may be practically applied to the early diagnosis, prevention and treatment of cervical cancer, since the kit can easily diagnose HPV infection, and can exactly determine the genotype of the HPV.



WO 01/68915 A1

**GENOTYPING KIT FOR DIAGNOSIS  
OF HUMAN PAPILLOMAVIRUS INFECTION**

5     BACKGROUND OF THE INVENTION

Field of the Invention

      The present invention relates to a genotyping kit for  
10     diagnosis of human papillomavirus(HPV) infection, more  
      specifically, to a genotyping kit for detecting human  
      papillomaviruses from clinical samples of infected patients  
      using a DNA chip, and a process for preparing the said DNA  
      chip and a method for diagnosis of HPV infection using the  
15     said genotyping kit.

Background of the Invention

      Uterine cancer includes cervical cancer, endometrial  
20     cancer, uterine sarcoma and the like. For cervical cancer,  
      approximately 450,000 new cases occur worldwide each year  
      and approximately 6,000 in Korea. Since the occurrence of  
      cervical cancer(including cervical intraepithelial  
      neoplasia) occupies 22.1% of total cancer cases in Korean  
25     women, the highest incidence with the second highest death  
      rate, the prevention, diagnosis and treatment of cervical  
      cancer are regarded as the most important issue in women's  
      health.

      Cervical cancer progresses through a precancerous  
30     stage, cervical intraepithelial neoplasia(CIN) known to be  
      mainly caused by human papillomavirus(HPV) infection.  
      Especially, infection by particular types of HPV raises the  
      possibility of developing invasive disease. Over 70  
      genotypes of HPV have been identified since the recognition  
35     of HPV as the main etiological factor for cervical cancer.  
      Certain HPV genotypes were selectively found in the lesions  
      of specific location or progression stage, which rendered

the biological diversity of HPV infection realized. Among the HPV genotypes detected in the anogenital area, over 10 genotypes have been classified as the high-risk group that are associated with an elevated risk for developing cervical cancer. Based on these findings, characterization of the biological differences of HPV infection is considered to be of significant importance to the diagnosis and prevention of cervical cancer.

For the diagnosis of cervical cancer at its early stage, Pap smear test has been most commonly used which is a cytological test performed as follows: old cells removed from the outermost layer of cells from the surface of the cervix are stained and examined for histopathological characteristics of HPV infection including koilocytosis, formation of perinuclear halo in the epithelial cells. However, due to the low diagnostic efficiency(1 - 15%) of Pap test together with other limitations, additional methods such as colposcopy are necessary for more dependable diagnosis. Colposcopic screening can detect HPV infection up to 70% but has disadvantages including high cost of the equipment, the need for skilled interpreters, and incapability of determining HPV genotypes to distinguish between the high-risk and low-risk infection. Therefore, efforts have been made continuously to develop techniques for the detection of HPV and identification of HPV genotypes to supplement conventional screening methods for cervical cancer and its precursors including Pap test.

The methods for detection of HPV and identification of HPV genotypes can be classified into two groups, i.e., direct detection of HPV DNA and detection of amplified HPV DNA. The methods for direct detection of HPV DNA include liquid hybridization(Hybrid Capture kit by Digene Diagnostics, Silver Spring, MD, USA, [www.digene.com](http://www.digene.com)), Southern blot and dot blot with HPV type-specific probes, filter in situ hybridization(FISH) and the like, and the methods for the detection of amplified DNA include type-specific PCR (polymerase chain reaction) and general-primer

PCR. In particular, genotype analyses of amplified HPV DNA by general primer sets are commonly performed by employing dot blot hybridization, microtiter plate hybridization, or line probe assay. Among these methods, liquid hybridization by Hybrid Capture and line probe assay following general-primer PCR have been considered most suitable for diagnostic purposes. The line probe assay can detect about 20 different HPV genotypes by immobilized oligonucleotide probes on a nitrocellulose membrane, however, it lacks reliability due to low sensitivity and difficulties in data interpretation. Commercialized Hybrid Capture kit can detect HPV DNA in clinical samples without PCR amplification and distinguish between high-risk and low-risk HPV groups. However, the fact that Hybrid Capture kit cannot identify the genotypes of infecting HPV limits accurate risk determination since the risk factor amongst the high-risk HPV is not the same, in other words, intermediate-risk types are included in the high-risk group. Moreover, the use of RNA probe may pose low stability of the kit, and also possibility of contamination cannot be excluded.

Under these circumstances, there have been strong reasons for exploring and developing a simple and accurate method for detection of HPV infection and identification of the genotype of infecting HPV.

#### Summary of the Invention

The present inventors have tried to detect HPV infection and identify the types of HPV by way of genotyping DNA from clinical samples and prepared an HPV genotyping kit comprising a DNA chip with probes that have nucleotide sequences complementary to the DNA of HPV, primers for amplifying DNA obtained from clinical samples by PCR, and means for labeling amplified DNA hybridized to the probes of the said DNA chip, and successfully detected HPV infection and identified genotypes of infecting HPV by

the aid of the genotyping kit in a simple and accurate manner.

5 A primary object of the present invention is, therefore, to provide a genotyping kit for diagnosis of HPV infection.

The other object of the invention is to provide a process for preparing the DNA chip contained in the HPV genotyping kit.

10 Another object of the invention is to provide a method for diagnosis of HPV infection using the HPV genotyping kit.

#### Brief Description of the Drawings

15

The above and the other objects and features of the present invention will become apparent from the following description given in the conjunction with the accompanying drawings, in which:

20

Figure 1 is a schematic representation of the types and positions of the probes on the DNA chip.

Figure 2a is a photograph showing the result of HPV 16 DNA analysis.

25 Figure 2b is a photograph showing the result of HPV 18 DNA analysis.

Figure 2c is a photograph showing the result of HPV 31 DNA analysis.

30 Figure 2d is a photograph showing the result of HPV 33 DNA analysis.

Figure 2e is a photograph showing the result of HPV 35 DNA analysis.

Figure 2f is a photograph showing the result of HPV 39 DNA analysis.

35 Figure 2g is a photograph showing the result of HPV 45 DNA analysis.

Figure 2h is a photograph showing the result of HPV

51 DNA analysis.

Figure 2i is a photograph showing the result of HPV  
52 DNA analysis.

Figure 2j is a photograph showing the result of HPV  
5 56 DNA analysis.

Figure 2k is a photograph showing the result of HPV  
58 DNA analysis.

Figure 2l is a photograph showing the result of HPV  
59 DNA analysis.

10 Figure 2m is a photograph showing the result of HPV  
66 DNA analysis.

Figure 3a is a photograph showing the result of HPV 6  
DNA analysis.

Figure 3b is a photograph showing the result of HPV  
15 11 DNA analysis.

Figure 3c is a photograph showing the result of HPV  
34 DNA analysis.

Figure 3d is a photograph showing the result of HPV  
40 DNA analysis.

20 Figure 3e is a photograph showing the result of HPV  
42 DNA analysis.

Figure 3f is a photograph showing the result of HPV  
44 DNA analysis.

Figure 4a is a photograph showing the result of DNA  
25 analysis of sample number 43 using the DNA chip of the  
invention.

Figure 4b is a photograph showing the result of DNA  
analysis of sample number 46 using the DNA chip of the  
invention.

30 Figure 4c is a photograph showing the result of DNA  
analysis of sample number 47 using the DNA chip of the  
invention.

Figure 4d is a photograph showing the result of DNA  
analysis of sample number 51 using the DNA chip of the  
35 invention.

Figure 4e is a photograph showing the result of DNA  
analysis of sample number 52 using the DNA chip of the

invention.

Figure 4f is a photograph showing the result of DNA analysis of sample number 53 using the DNA chip of the invention.

5        Figure 4g is a photograph showing the result of DNA analysis of sample number 54 using the DNA chip of the invention.

10       Figure 4h is a photograph showing the result of DNA analysis of sample number 57 using the DNA chip of the invention.

Figure 4i is a photograph showing the result of DNA analysis of sample number 95 using the DNA chip of the invention.

15       Figure 4j is a photograph showing the result of DNA analysis of sample number 107 using the DNA chip of the invention.

Figure 4k is a photograph showing the result of DNA analysis of sample number 115 using the DNA chip of the invention.

20       Figure 4l is a photograph showing the result of DNA analysis of sample number 124 using the DNA chip of the invention.

#### DETAILED DESCRIPTION OF THE INVENTION

25

The genotyping kit of the invention for diagnosis of human papillomavirus(HPV) infection comprises: a DNA chip with probes that have nucleotide sequences complementary to DNA of HPV; primers for amplifying DNA obtained from clinical samples by PCR; and, means for labeling amplified DNA hybridized with the probes of the said DNA chip. The DNA chip may further comprise position markers to locate probes, and staining or labeling is performed by using means for labeling comprising preferably biotin-binding material, most preferably, streptavidin-R-phycoerythrin which is a conjugate of a fluorophore and a protein with biotin-binding sites.

30

35



The process for preparing DNA chip contained in the said HPV genotyping kit comprises the steps of: preparing 5' terminal amine-linked DNA probes which have nucleotide sequences complementary to DNA of HPV; affixing the DNA probes thus prepared to an aldehyde-derivatized solid surface; and, reducing excessive aldehydes not reacted with amine.

The process for preparing DNA chip of the invention is described in more detail by the following steps.

Step 1: Preparation of probes

5' terminal amine-linked DNA probes that have nucleotide sequences complementary to the DNA of HPV are prepared: The nucleotide sequences of the probes are designed and synthesized to have nucleotide sequences complementary to the DNA of HPV, preferably the L1 region of HPV DNA, and the probes are prepared by linking amine group at 5' terminal of the nucleotide sequences which enables the probes to bind to aldehyde-derivatized solid surface.

Step 2: Affixture of probes

DNA probes prepared in Step 1 are affixed to an aldehyde-derivatized surface of a solid support, preferably glass. The probes are affixed to the surface of solid support via Schiff's base reaction between an aldehyde group on the surface of solid support and an amine group at 5' terminal of the probe under an environment of 30 to 40°C and 70 to 100% humidity, while controlling the concentration of probes in a range of preferably 100 to 300 pmol/ $\mu$ l, more preferably 200 pmol/ $\mu$ l.

Step 3: Preparation of DNA chip

Excessive aldehydes not reacted with amine on the solid surface are reduced by employing a reducing agent of NaBH<sub>4</sub> (sodium borohydride), finally to prepare DNA chip.

5

The method for diagnosis of HPV infection using HPV genotyping kit of the invention comprises the steps of: amplifying DNA obtained from clinical samples by PCR with primers of the HPV genotyping kit; applying the amplified DNA to the DNA chip to hybridize the amplified DNA with DNA probes of the DNA chip; and, detecting DNA bound on the surface of the DNA chip after labeling hybridized DNA.

The method for diagnosis of HPV infection using HPV genotyping kit of the invention is further illustrated by the following steps.

Step 1: Amplification of sample DNA

DNA obtained from clinical samples is amplified using the primers of HPV genotyping kit, where polymerase chain reaction (PCR) employing biotin-16-dUTP is carried out to give biotin-containing amplified DNA.

Step 2: Hybridization

Amplified DNA thus obtained is applied to the DNA chip of HPV genotyping kit and hybridized with the probes of the DNA chip.

30

Step 3: Detection

The amplified sample DNA hybridized with the probes are labeled with means for labeling and detected with a confocal laser scanner: Streptavidin-R-phycoerythrin is preferably used as means for labeling which is a conjugate of a fluorophore with a high extinction coefficient and a

35

protein with 4 biotin-binding sites, which enables high sensitivity detection of hybridized spots on the DNA chip by the confocal laser scanner.

5 HPV genotyping kit of the invention is an implement that can detect HPV infection in a simple and accurate manner, as well as identify the types of infecting HPV, therefore, it may contribute to early diagnosis, prevention and treatment of cervical cancer.

10

The present invention is further illustrated in the following examples, which should not be taken to limit the scope of the invention. Particularly, though DNA chip with 19 probes was prepared in the Examples described below, it is to be understood that the present invention is not limited by types and numbers of probes, but DNA chips using nucleotide sequences derived from HPV DNA and any variety of detection kits using the said DNA chips are intended to be included within the scope of the invention.

20

Example 1: Preparation of DNA chip

Prevalent HPV types including 13 high-risk types(HPV type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66) and 6 low-risk types(HPV type 6, 11, 34, 40, 42, 44) were selected, and genotype-specific probe for each HPV type possessing amine group at 5' terminal of the sequence was prepared for the detection of HPV genotypes. The nucleotide sequence of each probe is as follows:

30 HPV 16: 5'-gtcattatgtgctgccatatctacttcaga-3'(SEQ ID NO: 1),  
HPV 18: 5'-tgcttctacacagtctcctgtacctgggca-3'(SEQ ID NO: 2),  
HPV 31: 5'-tgtttgctgctgcaattgcaaacagtgatac-3'(SEQ ID NO: 3),  
HPV 33: 5'-tttatgcacacaagtaactagtgacagtac-3'(SEQ ID NO: 4),  
HPV 35: 5'-gtctgtgtgttctgctgtgtcttctagtga-3'(SEQ ID NO: 5),  
35 HPV 39: 5'-tctacctctatagagtcttccataccttct-3'(SEQ ID NO: 6),  
HPV 45: 5'-acacaaaatcctgtgccaaagtacatatgac-3'(SEQ ID NO: 7),  
HPV 51: 5'-agcactgccactgctgcggtttccccaaca-3'(SEQ ID NO: 8),

HPV 52: 5'-tgctgagggttaaaaaggaaagcacatataa-3'(SEQ ID NO: 9),  
HPV 56: 5'-gtactgctacagaacagttaagtaaataatg-3'(SEQ ID NO: 10),  
HPV 58: 5'-attatgcactgaagtaactaaggaaggtac-3'(SEQ ID NO: 11),  
HPV 59: 5'-ctgtgtgtgcttctactactgcttctattc-3'(SEQ ID NO: 12),  
5 HPV 66: 5'-ctattaatgcagctaaaagcacattaacta-3'(SEQ ID NO: 13),  
HPV 6: 5'-atccgtaactacatcttccacatacaccaa-3'(SEQ ID NO: 14),  
HPV 11: 5'-atctgtgtctaaatctgctacatacactaa-3'(SEQ ID NO: 15),  
HPV 34: 5'-tacacaatccacaagtacaaatgcaccata-3'(SEQ ID NO: 16),  
HPV 40: 5'-gctgccacacagtccccacaccaacccca-3'(SEQ ID NO: 17),  
10 HPV 42: 5'-ctgcaacatctggtgatacatatacagctg-3'(SEQ ID NO: 18),  
HPV 44: 5'-gccactacacagtcccctccgtctacatat-3'(SEQ ID NO: 19),

DNA chip was prepared as follows: each probe prepared  
above was dissolved in 3X SSC(45mM sodium citrate, 0.45M  
15 NaCl, pH 7.0) at a concentration of 200 pmol/ $\mu$ l, and  
spotted onto an aldehyde-derivatized silylated slide(CSS-  
100, CEL, Houston, TX, USA) to form an array of spots with  
size of 150/ $\mu$ m at 300/ $\mu$ m spacing between spots using a  
microarrayer(GMS 417 Arrayer, TakaRa, Japan), followed by  
20 performing Schiff's base reaction under an environment of  
37°C and over 70% humidity for 4 hours. The slide was  
washed with 0.2%(w/v) sodium dodecyl sulfate(SDS), and with  
triple distilled water. Then, the slide was treated with  
NaBH<sub>4</sub> solution(0.1g NaBH<sub>4</sub>, 30ml phosphate buffered saline  
25 (PBS), 10ml ethanol) for 5 minutes to reduce excessive  
aldehydes not reacted with amine, followed by washing with  
triple distilled water and air-drying.

#### Example 2: Preparation of samples

30

In order to detect HPV infection in human cervical  
swabs, DNA was extracted from the said specimen and then  
purified. To test the adequacy of sample DNA, the said  
purified DNA was PCR amplified with beta-globin primers,  
35 PC03(5'-acacaactgtgttcactagc-3', SEQ ID NO: 20) and 5'-  
biotin linked-PC04(5'-caacttcattccacgttcacc-3', SEQ ID NO:  
21). The DNA samples which reveal beta-globin DNA

amplification were selected and used for further analyses of HPV DNA.

As HPV DNA standards, plasmid DNA comprising HPV sequence obtained from the following distributors were used: HPV types 6, 11, 40, 45, and 51 from Dr. Ethel-Michele de Villiers, Angewandte Tumorvirologie, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 242, 69009 Heidelberg, Germany; HPV types 35, 44, and 56 from Dr. Attila Lörincz, Vice President, R&D and Scientific Director, Digene Diagnostics, Inc., 2301-B Broadburch Drive, Silver Spring, MD 20904, USA; HPV types 42, 58, and 59 from Dr. Toshihiko Matsukura, Department of Pathology and Laboratory of Pathology, AIDS Research Center, National Institute of Infectious Disease, Tokyo 162, Japan; HPV types 33, 34, 39, 52, and 66 from Dr. Gérard Orth, Unité Mixte Institut Pasteur/INSERM(U. 190), Institut Pasteur, 25 rue du Docteur Roux, 75724 Paris Cedex 15, France.

Additionally, DNA extracted and purified from following cell lines were used as positive controls: SiHa cell line(HPV 16, KCLB 30035, Human squamous carcinoma, cervix) and HeLa cell line(HPV 18, KCLB 10002, Human epithelial carcinoma, cervix) which were purchased from Korean Cell Line Bank(Seoul National University, College of Medicine, Seoul, Korea).

Selected sample DNA described above were PCR amplified using the following primer sets: GP5'(5'-tttggttactgtggttagatactac-3', SEQ ID NO: 22) and biotin-linked GP6', Bio-GP6'(5'-Biotin-gaaaaataaaactgtaaatcatattc-3', SEQ ID NO: 23), and, GP5d'(5'-tttkttachgtkgtgdgatacyac-3', SEQ ID NO: 24) and GP6d'(5'-gaaahataaaytgyaadtcataytac-3', SEQ ID NO: 25). The modified primer set GP5d'/GP6d' was developed to facilitate PCR amplification of HPV DNA from clinical samples. In describing the nucleotide sequence, 'k' is employed to mean 'g' or 't', 'h' is 't', 'a', or 'c', 'd' is 'a', 't', or 'g', and 'y' is 't' or 'c'.

Example 2-1: Preparation of positive control samples

To obtain biotin-labeled amplified DNA samples, HPV 16 and HPV 18 DNA purified above were amplified by PCR with  
5 primers, GP5<sup>+</sup> and Bio-GP6<sup>+</sup>. PCR was performed in a 50  $\mu$ l of reaction mixture containing PCR buffer(50 mM KCl, 4 mM MgCl<sub>2</sub>, 10 mM Tris-HCl, pH 8.3), 0.1  $\mu$ g of DNA, 4.5 mM MgCl<sub>2</sub>, 50 pmol of each primer, 40  $\mu$ M each of dATP, dCTP, dGTP(Pharmacia), 30  $\mu$ M of dTTP(Pharmacia), 10  $\mu$ M biotin-  
10 16-dUTP(Boehringer Mannheim, Germany) and 1 unit of Taq polymerase(TaKaRa, Japan) with 40 cycles of denaturation for 1 min at 94°C, primer annealing for 2 min at 40°C, and extension for 1 min 30sec at 72°C.

Example 2-2: Preparation of HPV standards

Biotin-linked amplified HPV DNA samples were prepared analogously as in Example 2-1, except for employing  
20 templates of various HPV plasmids described above.

Example 2-3: Preparation of sample DNA from clinical samples

Biotin-linked amplified DNA samples were obtained  
25 analogously as in Example 2-1, except that DNA obtained from uterine cervical swabs were used as templates, GP5d<sup>+</sup> and GP6d<sup>+</sup> were employed as primers, and PCR was performed with 40 cycles of denaturation for 1 min at 94°C, primer annealing for 2 min at 55°C, and extension for 1 min 30 sec  
30 at 72°C.

Example 3: Detection of HPV infection using DNA chip

Amplified DNA samples obtained in Example 2 were  
35 applied to the DNA chip prepared in Example 1, and hybridization was carried out in a hybridization reaction chamber made up of the Cover slip(GRACE Bio-Labs, USA,

PC4L-1.0) with 100  $\mu\text{l}$  capacity.

As for the quantity of hybridization reaction samples, 10  $\mu\text{l}$  each of amplified product was used for positive controls and plasmid DNA, and a mixture of 10  $\mu\text{l}$  of HPV amplified product and 5  $\mu\text{l}$  of beta-globin amplified product was used for DNA obtained from cervical swabs. The said reaction samples were denatured by adding 3N NaOH solution(10% v/v) and standing for 5 min at room temperature, and neutralized by adding 1 M Tris-HCl(pH 7.2, 5% v/v) followed by 3N HCl(10% v/v) and cooling for 5 min on ice. The samples were then mixed with a hybridization solution made up of 6X SSPE(saline-sodium phosphate-EDTA buffer, Sigma Chemical Co., St. Louis, MO, USA) and 0.2% SDS(sodium dodecyl sulfate), and applied onto the DNA chip. Hybridization reaction was carried out for 2 hours at 40°C, followed by washing with 3X SSPE for 2 min, 1X SSPE for 2 min, and air-drying at room temperature. The DNA chip hybridized with sample DNA was stained with a mixture of 5  $\mu\text{l}$  of streptavidin-R-phycoerythrin conjugate(50  $\mu\text{g}/\text{ml}$ ) and 95  $\mu\text{l}$  of 3X SSPE for 25 min, washed with 1X SSPE, and then analyzed for fluorescent signals(extinction 480 nm, emission >520 nm) by using a confocal laser scanner(GMS 418 Array Scanner, TaKaRa, Japan)(see: Figure 1, Figures 2a-2m, 3a-3f, and 4a-4l). Figure 1 is a schematic representation of the type and position of the probes on DNA chip: each number indicates each HPV probe, 'bg' indicates beta-globin probe placed to verify proper performance of hybridization reaction, 'M' indicates position marker for locating probes, open circles( $\bigcirc$ ) indicate HPV and beta-globin probe-affixed positions, and closed circles( $\bullet$ ) indicate positions of 'M'. Figures 2a-2m are photographs showing the results of high-risk group HPV DNA analyses using HPV plasmids and cervical cancer cell lines: Figure 2a is a photograph showing the result of HPV 16 DNA analysis, Figure 2b, the result of HPV 18 DNA analysis, Figure 2c, the result of HPV 31 DNA analysis, Figure 2d, the result of HPV 33 DNA analysis, Figure 2e, the result of HPV 35 DNA analysis, Figure 2f,

the result of HPV 39 DNA analysis, Figure 2g, the result of HPV 45 DNA analysis, Figure 2h, the result of HPV 51 DNA analysis, Figure 2i, the result of HPV 52 DNA analysis, Figure 2j, the result of HPV 56 DNA analysis, Figure 2k, the result of HPV 58 DNA analysis, Figure 2l, the result of HPV 59 DNA analysis, and Figure 2m, the result of HPV 66 DNA analysis. Figures 3a-3f are photographs showing the results of low-risk group HPV DNA analyses using HPV plasmids: Figure 3a is a photograph showing the results of HPV 6 DNA analysis, Figure 3b is a photograph showing the result of HPV 11 DNA analysis, Figure 3c, the result of HPV 34 DNA analysis, Figure 3d, the result of HPV 40 DNA analysis, Figure 3e, the result of HPV 42 DNA analysis, and Figure 3f, the result of HPV 44 DNA analysis. As shown in Figures 2a-2m and Figures 3a-3f, hybridization signals produced by the amplified DNA of HPV plasmid standards and HPV positive controls(cervical cancer cell lines) were observed clearly on the corresponding probes without significant cross-hybridization.

20

Example 4: Detection of HPV infection in clinical samples using DNA chip

In order to examine the accuracy and efficiency of diagnosis by the DNA chip of the invention, clinical samples were PCR amplified with primers comprising nucleotide sequences set forth in SEQ ID NO: 20-25, and then, for proper samples, diagnostic procedure using the DNA chip was performed to detect HPV infection as well as to determine the type of the infection.

DNA isolated from 124 specimens from uterine cervix were amplified using the method described in Example 2-3, and analyzed for HPV infection by using the DNA chip of the invention as described in Example 3. The above 124 isolated DNA were subjected to PCR-RFLP(Restriction Fragment Length Polymorphism) assay in which DNA was amplified, treated with restriction enzyme Ava II, Afa I,



Bgl II, Acc I or Ava I, and then the pattern of fragmentation produced by the restriction enzymes was analyzed to determine 6 types of HPV infection (HPV 16, 18, 31, 33, 52 and 58). The results of PCR-RFLP were confirmed by employing type-specific PCR technique (see: Hwang, T., J. Kor. Med. Sci., 15:593-599, 1999; Fujinaga, Y. et al., J. General Virology, 72:1039-1044, 1991). The results of the two methods, DNA chip of the invention and PCR-RFLP followed by type-specific PCR, were compared to determine diagnostic efficiency of the DNA chip method (see: Figures 4a-4l, Table 1). Figures 4a-4l are photographs showing Examples of the results of DNA chip analyses of cervical swab specimens for HPV infection. As shown in Figures 4a-4l, detailed diagnoses of HPV infection in the clinical samples via accurate detection and genotyping of the infecting HPV were successfully accomplished by using the DNA chip of the invention. The degree of agreement of the two methods was also measured for the above 124 cases of clinical specimens (see: Table 1).

20

Table 1. Comparison of the detection/genotyping results obtained by the invented genotyping kit and PCR-RFLP assay of prior art

Sample Nos.	Detection/Genotyping by Genotyping Kit	Detection/Genotyping by PCR-RFLP Assay
34	-	-
35	-	-
36	-	HPV 16
37	HPV 16, 56	HPV 16
38	HPV 58	HPV 58
39	HPV 56, 58	HPV 33
40	HPV 16	HPV 16
41	HPV 16	HPV 18
42	HPV 16	HPV 16
43	HPV 16	HPV 16
46	HPV 16	HPV 16
47	HPV 16	HPV 16
48	HPV 33	HPV 33
49	HPV 33	HPV 33

50	HPV 51	HPV 18
51	HPV 16	HPV 16
52	HPV 33	HPV 33
53	HPV 58	HPV58
54	HPV 16, 18	HPV 16, 18
57	HPV 58	HPV 58
58	HPV 33	HPV 33
59	HPV 18	HPV 18
60	HPV 18	HPV 18
62	HPV 39	-
63	HPV 35	HPV 35
64	-	-
65	HPV 58	HPV 58
66	-	-
68	No typing	HPV 52, 58
69	No typing	-
70	HPV 16	HPV 16
71	HPV 16	HPV 16
72	-	-
73	HPV 16	HPV 16
75	-	HPV 16
76	HPV 16	HPV 16
77	HPV 18	HPV 16
78	HPV 16	HPV 16
79	HPV 33, 35	HPV 33
80	HPV 33	HPV 33
81	HPV 16	HPV 16
82	HPV 16	HPV 16
83	-	HPV 33
84	HPV 16	HPV 16
85	HPV 16	HPV 16
86	HPV 16	HPV 16
87	HPV 16	HPV 16
88	HPV 16	HPV 16
89	HPV 58	HPV 58
90	HPV 16	HPV 16
91	HPV 16	HPV 16
92	HPV 16	HPV 16
93	HPV 16	HPV 16
94	HPV 16	HPV 16
95	HPV 16	HPV 16
96	-	HPV 33
97	HPV 16	HPV 16
98	HPV 16	HPV 16
99	HPV 16	HPV 16
100	HPV 16	HPV 16

101	HPV 16	HPV 16
102	HPV 16	HPV 16
103	HPV 16	HPV 16
104	HPV 16	HPV 16
105	HPV 16	HPV 16
106	HPV 16	HPV 16
107	HPV 16	HPV 16
108	HPV 16	HPV 16
109	HPV 16	HPV 16
110	HPV 16	HPV 16
111	HPV 18	HPV 18
112	HPV 16	HPV 16
113	HPV 16	HPV 16
114	HPV 16	HPV 16
115	HPV 31, 35	HPV 31
116	HPV 16	HPV 16
117	HPV 16	HPV 16
118	HPV 16	HPV 16
119	HPV 16	HPV 16
120	HPV 16	HPV 16
121	HPV 16	HPV 16
123	HPV 16	HPV 16
124	HPV 51	HPV 31

In Table 1, "no typing" indicates presence of HPV DNA after PCR amplification with HPV type undetermined by the specific method, and "-" indicates absence of HPV DNA after PCR amplification. As shown in Table 1, the results obtained by two methods under comparison were in a good accordance demonstrating the reliability of DNA chip analysis. Considering the simplicity and rapidity of procedure, together with convenient detection of diverse genotypes and multiple infection, DNA chip analysis is thought to be by far more advantageous than PCR-RFLP followed by type-specific PCR and other related methods. The accuracy of diagnosis by the DNA chip of the invention was calculated to be 96.5% and the reproducibility was 95% based on the above 124 cases, which are considered to be the subject of improvement upon completion of larger size case studies in progress. The FDA (Food and Drug Administration) approved Hybrid Capture kit increasingly

employed recently for fast diagnosis of HPV infection was reported to have 98% accuracy in detecting and distinguishing high- or low-risk HPV infection. DNA chip analysis has a competitive efficiency and an additional advantage of genotyping when compared with the Hybrid Capture assay. The above information indicates that diagnosis of HPV infection using the genotyping kit of the invention is superior in many aspects to the conventional methods employed for the same purpose.

As clearly illustrated and demonstrated as above, the present invention provides a genotyping kit for identifying genotypes of HPV from clinical samples of infected patients and a method for diagnosis of HPV infection by genotyping the infecting virus using the said genotyping kit. The HPV genotyping kit of the invention comprises: a DNA chip with probes that have nucleotide sequences complementary to DNA of HPV; primers for amplifying DNA obtained from clinical samples by PCR; and, means for labeling amplified DNA hybridized with the probes of the said DNA chip. The method for diagnosis of HPV infection using the said HPV genotyping kit comprises the steps of: amplifying DNA obtained from clinical samples by PCR with primers of the kit; applying the amplified DNA to DNA chip to hybridize the amplified DNA with the probes of the DNA chip; and, detecting DNA bound on the surface of the DNA chip after labeling DNA hybridized with the probes of the DNA chip with means of labeling of the HPV genotyping kit. HPV genotyping kit of the invention is an implement that can detect HPV infection in a simple and accurate manner, as well as identify the types of infecting HPV, therefore, it may contribute to early diagnosis, prevention and treatment of cervical cancer.

Various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing descriptions.

Such modifications are also intended to fall within the scope of the appended claims.

What is claimed is:

1. A Human Papillomavirus(HPV) genotyping kit which comprises:

- 5 (i) a DNA chip with probes that have nucleotide sequences complementary to DNA of HPV;  
(ii) primers for amplifying DNA obtained from clinical samples by PCR; and,  
(iii) means for labeling amplified DNA hybridized with  
10 the probes of the said DNA chip.

2. The HPV genotyping kit of claim 1 wherein the DNA chip further comprises position markers to locate probes.

- 15 3. The HPV genotyping kit of claim 1 wherein the primers are selected from the group consisting of GP5+ having Sequence ID No. 22, GP6+ having Sequence ID No. 23, GP5d+ having Sequence ID No. 24 and GP6d+ having Sequence ID No. 25.

20

4. The HPV genotyping kit of claim 1 wherein the means for labeling is a biotin-binding material.

- 25 5. The HPV genotyping kit of claim 4 wherein the biotin-binding material is streptavidin-R-phycoerythrin.

6. A process for preparing a DNA chip which comprises the steps of:

- 30 (i) preparing 5' terminal amine-linked DNA probes which have nucleotide sequences complementary to DNA of HPV;  
(ii) affixing the DNA probes thus prepared to an aldehyde-derivatized surface of solid support; and  
(iii) reducing excessive aldehydes not reacted with  
35 amine.

7. The process for preparing DNA chip of claim 6

wherein the concentration of probes which react with aldehyde-derivatized solid surface ranges from 100 to 300pmol/ $\mu$ l.

5           8. The process for preparing DNA chip of claim 6 wherein affixing DNA probes to aldehyde-derivatized solid surface is performed via Schiff's base reaction between amine and aldehyde groups under an environment of 30 to 40 C and 70 to 100% humidity.

10

          9. The process for preparing DNA chip of claim 6 wherein the reduction of aldehyde is performed by the aid of a reducing agent,  $\text{NaBH}_4$ .

15

          10. A method for diagnosis of HPV infection using a HPV genotyping kit which comprises the steps of:

          (i) amplifying DNA obtained from clinical samples by PCR with primers of HPV genotyping kit of claim 1 to give biotin-containing amplified DNA;

20

          (ii) applying the amplified DNA thus obtained to DNA chip of the HPV genotyping kit to hybridize the amplified DNAs with DNA probes of the DNA chip; and,

          (iii) detecting DNA bound on the surface of the DNA chip after labeling amplified DNA hybridized with the probes with means for labeling of the HPV genotyping kit.

25

          11. The method for diagnosis of HPV infection using a HPV genotyping kit of claim 10 wherein the amplification of DNA obtained from clinical samples is performed by PCR using biotin-16-dUTP.

30

35

1/16

58	○ ○ ●	16	○ ○ ●
59	○ ○ ●	18	○ ○ ●
66	○ ○ ●	31	○ ○ ●
bg	○ ○ ●	33	○ ○ ●
06	○ ○ ●	35	○ ○ ●
11	○ ○ ●	39	○ ○ ●
34	○ ○ ●	45	○ ○ ●
40	○ ○ ●	51	○ ○ ●
42	○ ○ ●	52	○ ○ ●
44	○ ○ ●	56	○ ○ ●

Fig. 1

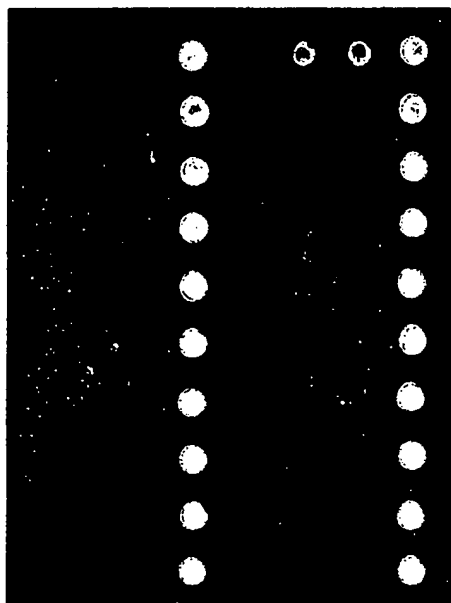


Fig. 2a



2/16

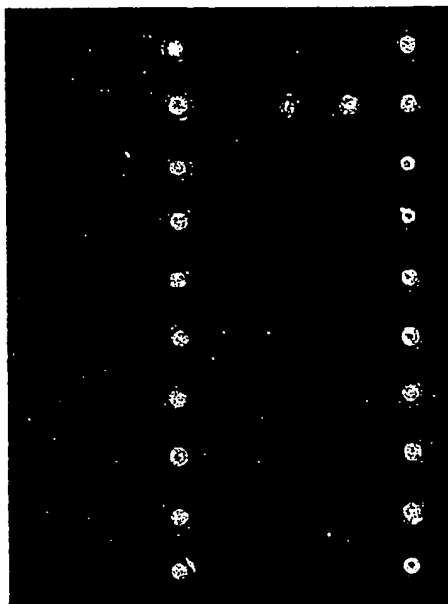


Fig. 2b

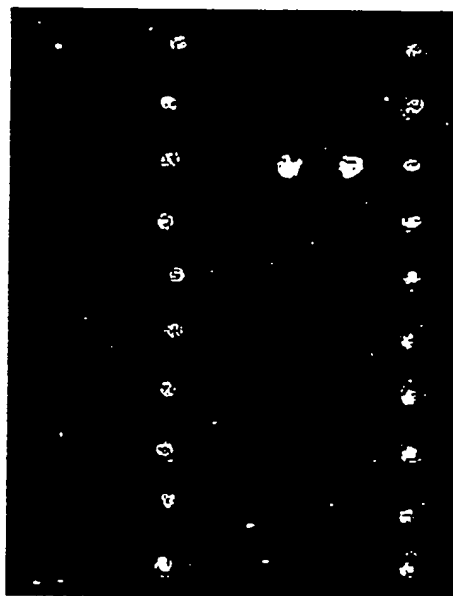


Fig. 2c

3/16

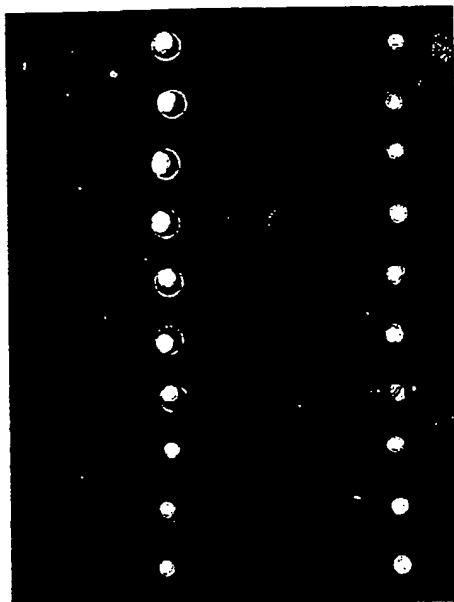


Fig. 2d

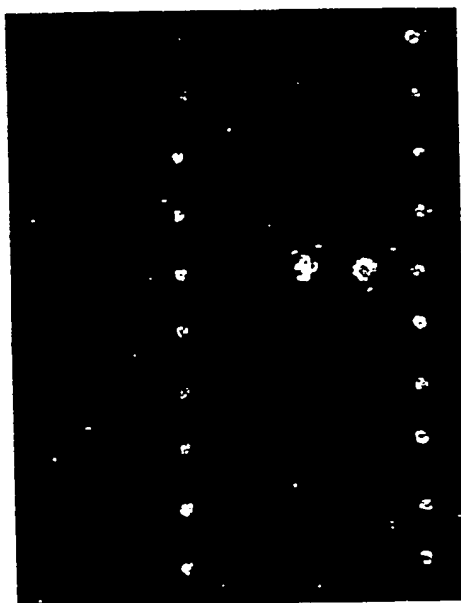


Fig. 2e

4/16

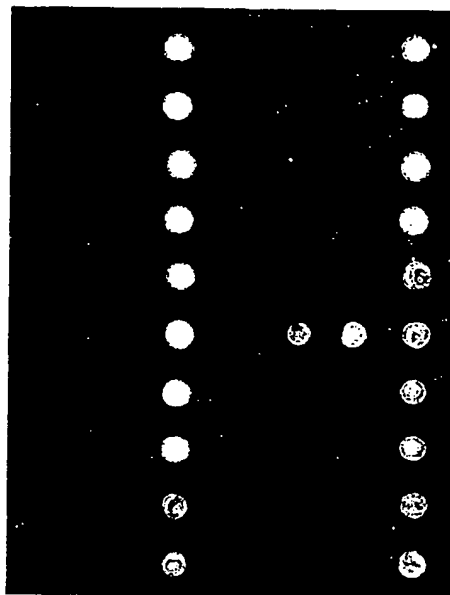


Fig. 2f

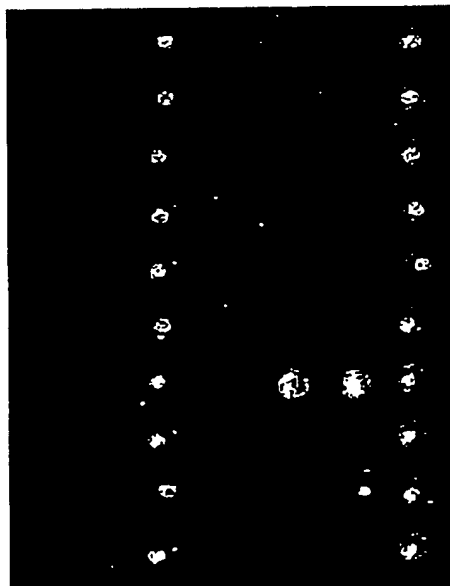


Fig. 2g

5/16

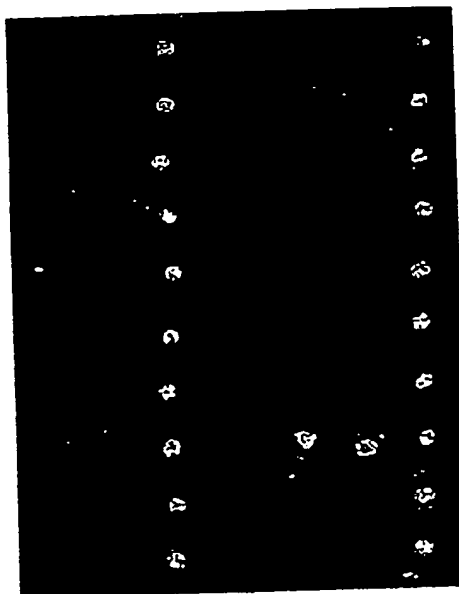


Fig. 2h

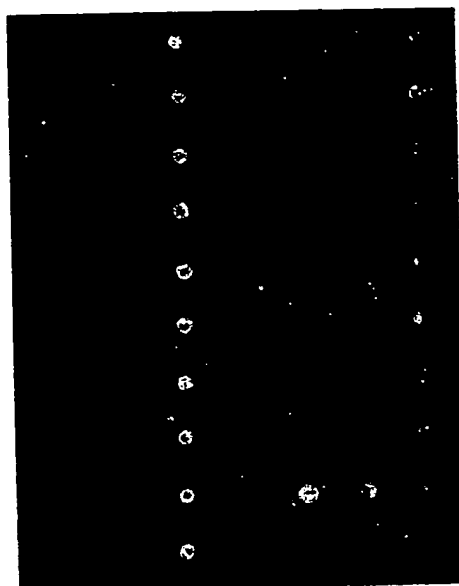


Fig. 2i

6/16

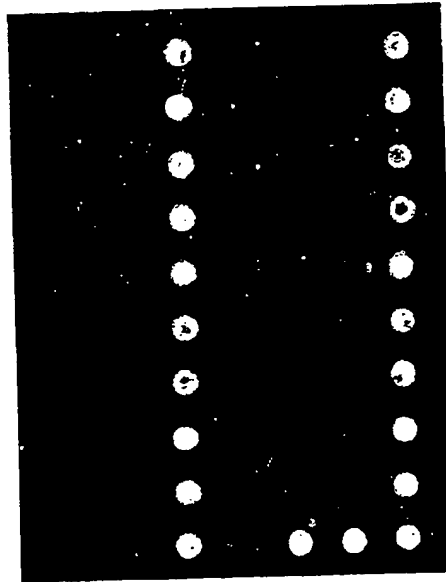


Fig. 2j

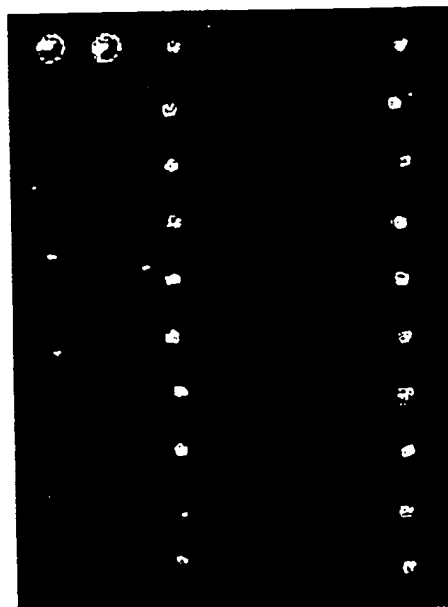


Fig. 2k

7/16

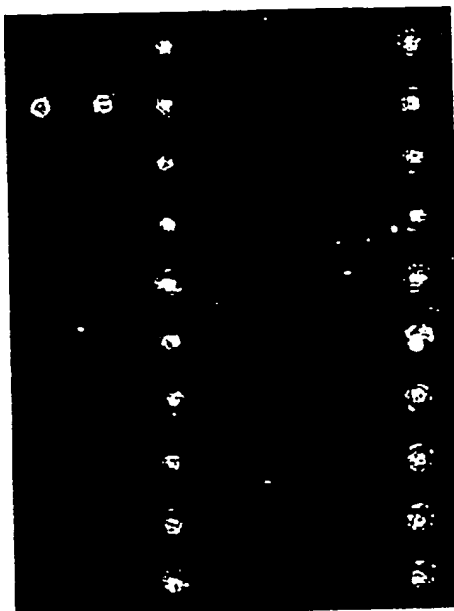


Fig. 2l

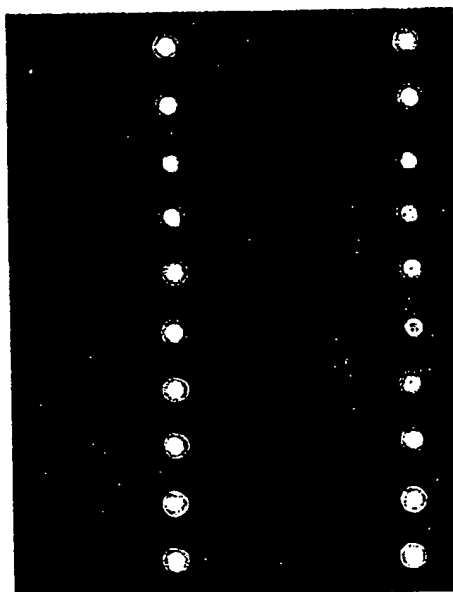


Fig. 2m

8/16

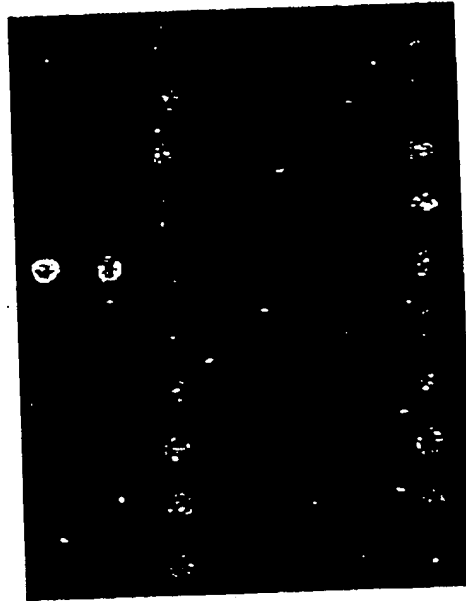


Fig. 3a

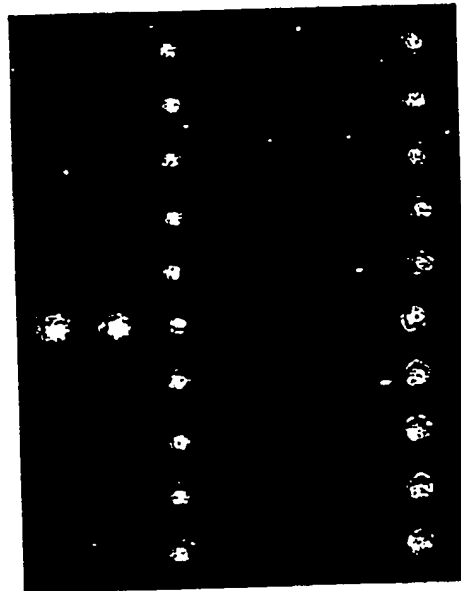


Fig. 3b

9/16

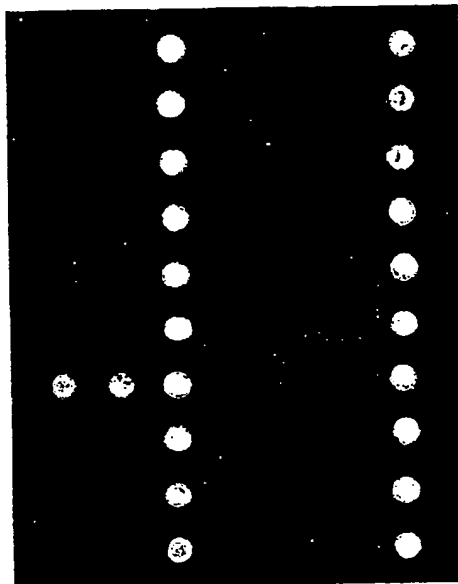


Fig. 3c

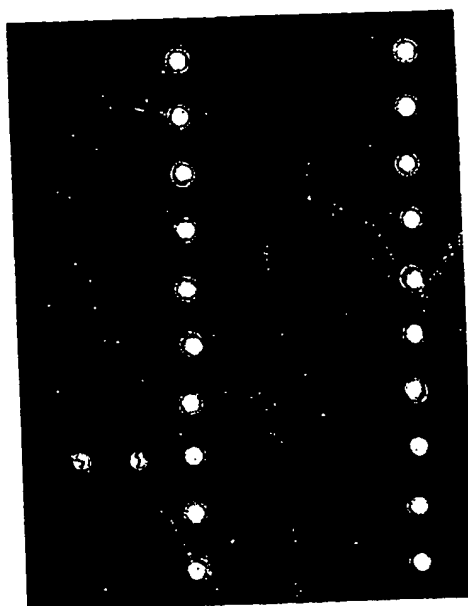


Fig. 3d



10/16

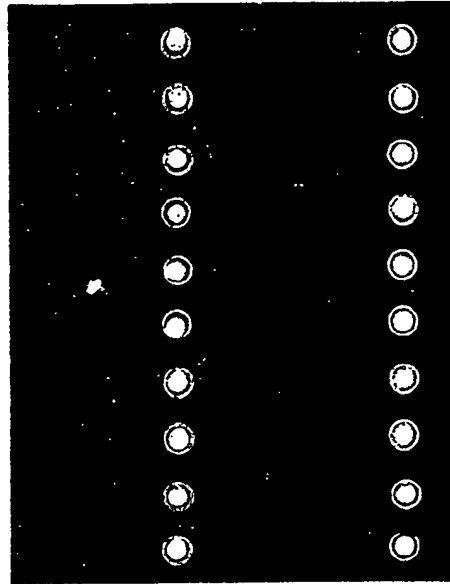


Fig. 3e

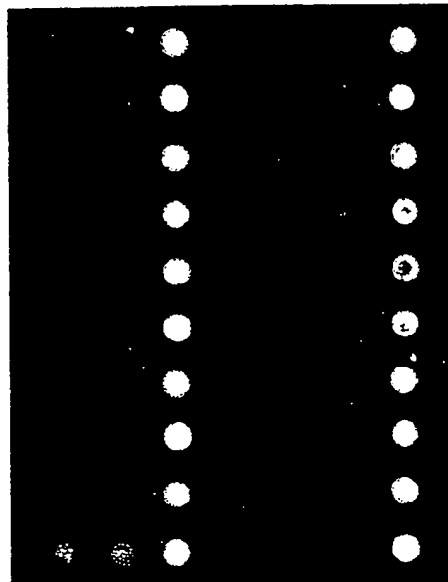


Fig. 3f

11/16

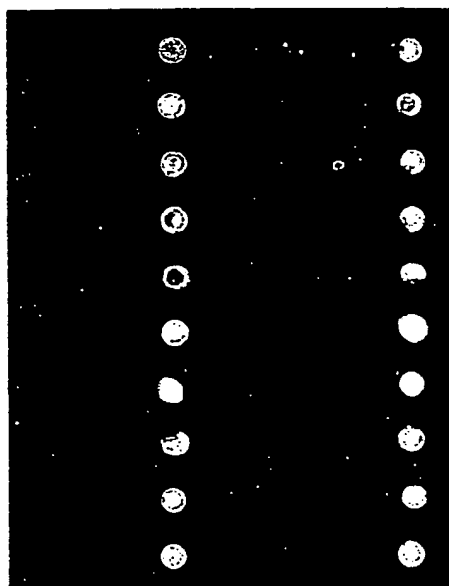


Fig. 4a

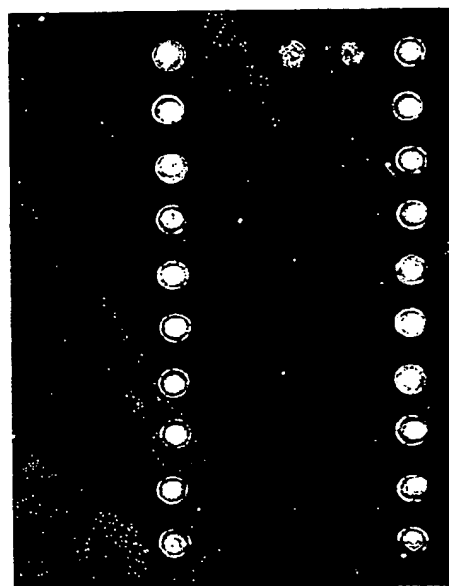


Fig. 4b

12/16

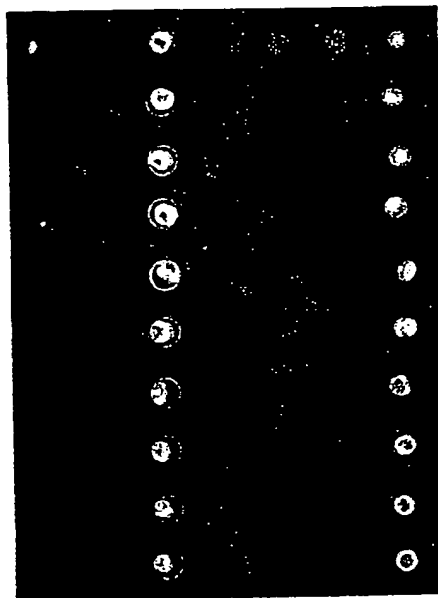


Fig. 4c

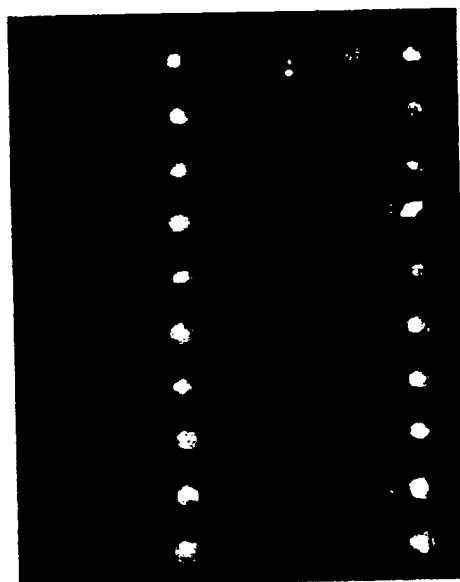


Fig. 4d

13/16

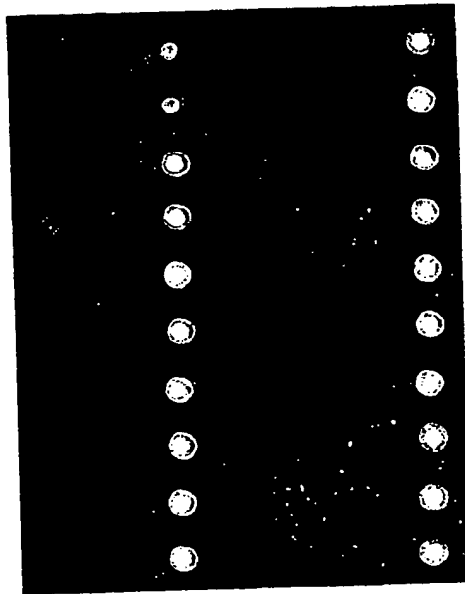


Fig. 4e

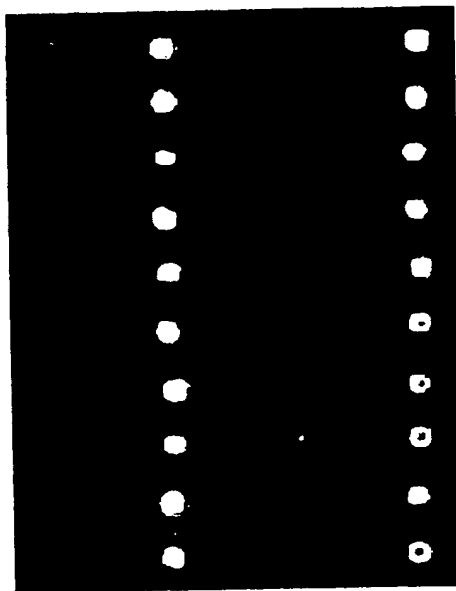


Fig. 4f

14/16

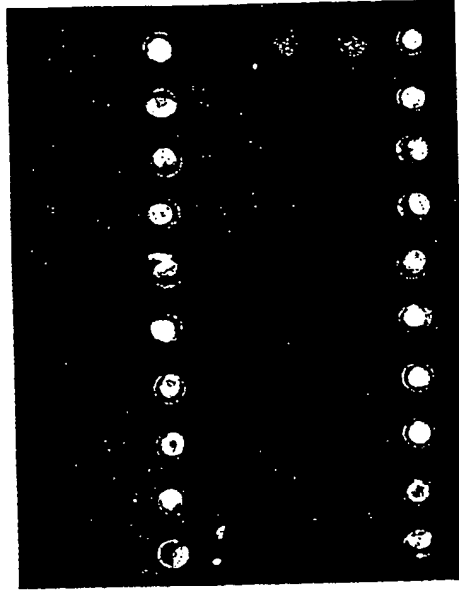


Fig. 4g

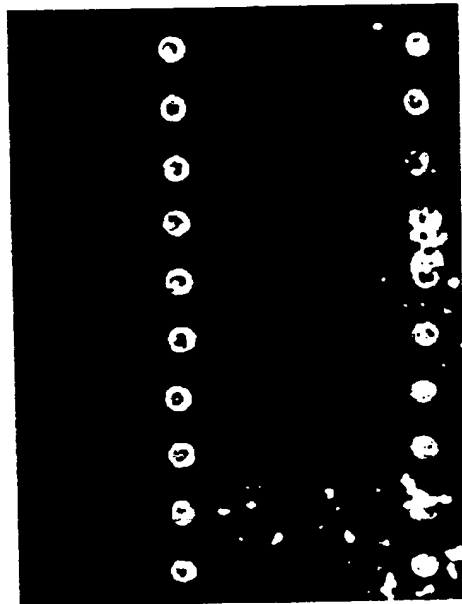


Fig. 4h

15/16

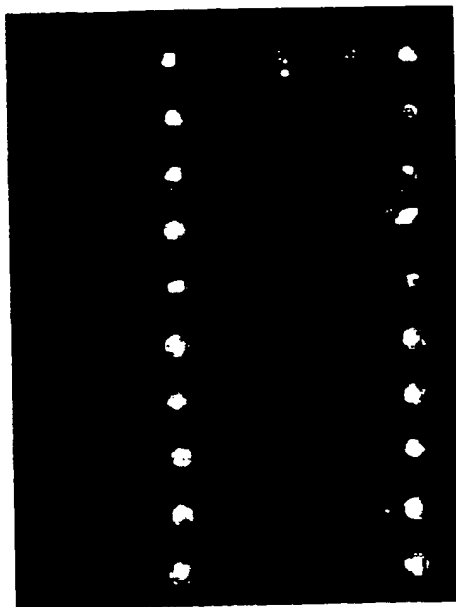


Fig. 4i

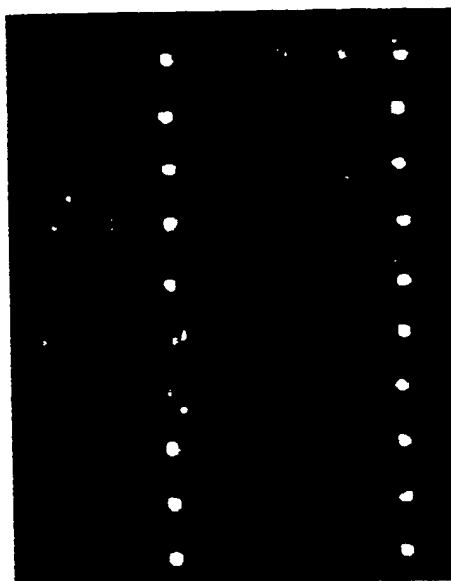


Fig. 4j

16/16

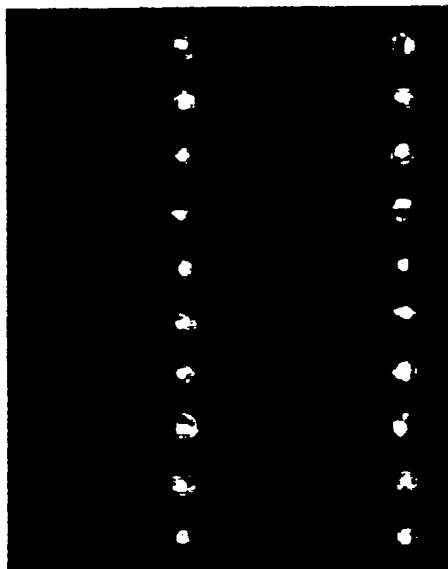


Fig. 4k

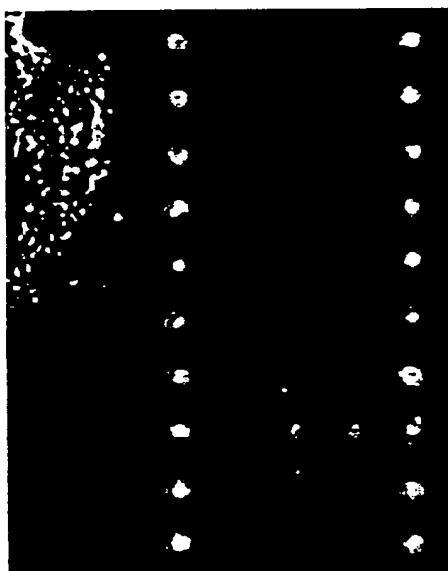


Fig. 4l

Sequence Listing

<110> BIOMEDLAB COOPERATION

<120> Genotyping kit for diagnosis of human papillomavirus infection

<130> P0066/BML/PCT

<150> KR 10-2000-0013161

<151> 2000-03-15

<160> 25

<170> KOPATIN 1.5

<210> 1

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> primer HPV16

<400> 1

gtcattatgt gctgccatat ctacttcaga

30

<210> 2

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> primer HPV18



<400> 2  
tgcttctaca cagtcctcctg tacctgggca

30

<210> 3  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer HPV31

<400> 3  
tgtttgtgct gcaattgcaa acagtgatac

30

<210> 4  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer HPV33

<400> 4  
tttatgcaca caagtaacta gtgacagtac

30

<210> 5  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer HPV35

<400> 5  
gtctgtgtgt tctgtgtgt cttctagtga

30

<210> 6  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer HPV39

<400> 6  
tctacctcta tagagtcttc cataccttct

30

<210> 7  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer HPV45

<400> 7  
acacaaaatc ctgtgccaag tacatatgac

30

<210> 8  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer HPV51

<400> 8  
agcactgcc a ctgctgcggt ttccccaaca

30

<210> 9  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer HPV52

<400> 9  
tgctgaggtt aaaaaggaaa gcacataaa

30

<210> 10  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer HPV56

<400> 10  
gtactgctac agaacagtta agtaaataatg

30

<210> 11  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer HPV58

<400> 11  
attatgcact gaagtaacta aggaaggtac

30

<210> 12  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer HPV59

<400> 12  
ctgtgtgtgc ttctactact gcttctattc

30

<210> 13  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer HPV66

<400> 13  
ctattaatgc agctaaaagc acattaacta

30

<210> 14  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer HPV6

<400> 14  
atccgtaact acatcttcca catcaccaa

30

<210> 15  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer HPV11

<400> 15  
atctgtgtct aaatctgcta catacactaa

30

<210> 16  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer HPV34

<400> 16  
tacacaatcc acaagtacaa atgcaccata

30

<210> 17  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer HPV40

<400> 17  
gctgccacac agtccccac accaacccea

30

<210> 18  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer HPV42

<400> 18  
ctgcaacatc tggatgatac tatacagctg

30

<210> 19  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer HPV44

<400> 19  
gccactacac agtccccctcc gtctacatat

30

<210> 20  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer PC03

<400> 20

acacaactgt gttcactagc

20

<210> 21

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> primer PC04

<400> 21

caacttcac cagttcacc

20

<210> 22

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> primer GP5+

<400> 22

tttggtactg tggtagatac tac

23

<210> 23

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> primer GP6+

<400> 23  
gaaaaataaa ctgtaaatca tattc

25

<210> 24  
<211> 23  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer GP5d+

<400> 24  
tttkttachg tkgtdgatac yac

23

<210> 25  
<211> 25  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer GP6d+

<400> 25  
gaaahataaa ytgyaadica taytc

25



## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/KR00/01213

**A. CLASSIFICATION OF SUBJECT MATTER****IPC7 C12Q 1/68**

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC7 C12Q 1/68

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
Korean patents and applications for inventions since 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Databases serviced by USPTO and ESPACE on their web sites  
Pubmed, PAJ

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5215882 A (ORTHO DIAGNOSTIC SYSTEMS, INC) 01 JUN 1993	1, 2, 4, 6-11
Y	US 5484699 A (ABBOTT LABORATORIES) 16 JAN 1996	1, 2, 4, 6-11
Y	EP 0774518 A2 (GEN-PROBE INC.) 21 MAY 1997	1, 2, 4, 6-11
Y	WO 9914377 A2 (DELFTS DIAGNOSTIC LABORATORY B.V.) 25 MAR 1999	1, 2, 4, 6-11
Y	WO 9817829 A2 (ABBOTT LABORATORIES) 30 APR 1998	1, 2, 4, 6-11

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

## \* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance  
 "E" earlier application or patent but published on or after the international filing date  
 "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)  
 "O" document referring to an oral disclosure, use, exhibition or other means  
 "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention  
 "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone  
 "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art  
 "&" document member of the same patent family

Date of the actual completion of the international search  
27 FEBRUARY 2001 (27.02.2001)

Date of mailing of the international search report  
28 FEBRUARY 2001 (28.02.2001)

Name and mailing address of the ISA/KR  
Korean Industrial Property Office  
Government Complex-Taejon, Dunsan-dong, So-ku, Taejon  
Metropolitan City 302-701, Republic of Korea  
Facsimile No. 82-42-472-7140

Authorized officer  
HAN, Hyun Sook  
Telephone No. 82-42-481-5596



**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International application No.

PCT/KR00/01213

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5215882 A	01.06.93	EP 435470 A1	03.07.91
US 5484699 A	16.01.96	JP4281791 A2 EP477972 A3	07.10.92 20.05.92
EP 0774518 A2	21.05.97	WO 97/18334	22.05.97

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR00/01213

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 10, 11  
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 10, 11 are related to methods of treatment of the human or animal body by surgery or by therapy, as well as diagnostic methods/PCT Rule 39.1(iv), the search has been carried out. The search has been based on the alleged effects of the compositions.

2. ☐ Claims Nos.:  
because they relate to part of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Search Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be established without effort justifying an additional fee, this Authority did not invite payment of any addition fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.